Pr tective P ptides N urotoxin f C. Botulinum

This application is a continuation-in-part of U.S. Patent Application 08,446,114, which issued as U.S. patent 6,287,566 B1 on September 11, 2001.

Field of the Invention:

This invention relates to immunization against toxic effect of neurotoxins of Clostridium bolutinum. Protective epitopes of the heavy chain of the neurotoxin of C. botulinum have been discovered. The invention also relates to preparation of protective immunotoxins of C. botulinum.

Background of the Invention:

Botulinum neurotoxin (BoNT) is one of the most potent toxins known to man. Ingestion or inhalation of toxin inhibits neurotransmitter release from synaptic vesicles, resulting in neuroparalysis and death. Seven serologically distinct forms of neurotoxin are produced by Clostridium botulinum. The toxin is synthesized as a 150 kDa precursor that is proteolytically nicked into two subunits. The light (L) chain, associated with the toxicity of BoNT, must be internalized in the cell in order to inhibit neurotransmitter release. It is linked by a disulfide bond to the heavy (H) chain, which mediates binding of the toxin to receptors located on the surface of the nerve cell. Although the heavy chain is required for BoNT to productively bind and enter the target cell, it is not toxic by itself.

The current pentavalent toxoid vaccine for botulism is composed of formalin-inactivated holotoxin. Although effective, this vaccine is difficult to manufacture. Furthermore, extensive treatment with formalin is required to inactivate the toxin. Prolonged treatment with formalin can affect the immunogenicity of protein antigens, and this may explain why certain lots of toxoid have been poorly immunogenic in the past.

There are several approaches that can be used to construct a new vaccine. One approach would be to express a non-toxigenic mutant of BoNT/A, as has already been done for other toxins. The advantage of this approach is that the immune response elicited by

the modified protein would most closely approximate the response elicited by the native toxin, because almost all of the native protein structure would still be intact. However, high level expression of the C fragment of tetanus toxin (TeTx) could not be achieved in E. coli when the native clostridial gene sequence was used. Based on this information, expression of BoNT might be predicted to be difficult, as well. Another approach is to construct a synthetic peptide-based vaccine. The advantage of this approach is that large quantities of synthetic peptide can be easily manufactured for use in a vaccine. However, studies with MAbs have indicated that many of the neutralizing epitopes located on BoNT are conformationally sensitive. This suggests that a peptide-based vaccine may not necessarily be able to induce neutralizing antibody responses due to its lack of conformational epitopes. A genetically engineered vaccine for botulism would eliminate many problems, since it could be expressed in a recombinant host at high levels and would not require treatment with formalin before incorporation into a vaccine.

Recent developments have made the construction of a genetically engineered BoNT vaccine possible. The gene for BoNT serotype A (BoNT/A) has been cloned and sequenced (Binz, et al., J. Biol. Chem. **265**:9153-9158.(1990), and the minimum length of the light chain needed to retain neurotoxicity has been defined (Kurazono, et al., J. Biol. Chem. **267**:14721-14729 (1992)). While construction of such a vaccine is feasible, there has not been a systematic attempt to identify the domain(s) of BoNT/A that would be required to elicit protective immunity. Immunization with a fragment corresponding to the C-terminal half of the heavy chain (H_C) has been shown to stimulate protective immunity, but more definitive identification of sequences that elicit protective immune response had not previously been described. Monoclonal antibodies directed against either light chain or heavy chain determinants had been shown to provide some passive protection to mice against a lethal exposure to BoNT, indicating that protective epitopes may exist on either chain. However, many of these pitopes appear to be conformationally sensitive, which suggests